IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants	:	Jan Zavada et al.	Technology Center: 1600
Serial No.	:	09/807,949	Art Unit: 1643
Filed	:	August 9, 2001	Confirmation No.: 9458
For	:	MN Gene and Protein	Examiner: Christopher H. Yaen

BRIEF ON APPEAL
(37 CFR § 41.37)

MAIL STOP APPEAL BRIEF-PATENTS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The following appeal brief is submitted pursuant to the Notice of Panel Decision from Pre-Appeal Brief Review mailed from the U.S. Patent and Trademark Office (PTO) on January 22, 2006, which Decision directed Appellants to proceed to file an appeal brief within one month of the mailing of said Decision.

TABLE OF CONTENTS

REAL PARTIES IN INTEREST

The real parties in interest for the subject application in addition to the inventors, include the assignee, which is the Institute of Virology of the Slovak Academy of Sciences, and the licensees which include Bayer Corporation, Siemens Medical Solutions Diagnostics, and Chiron Corporation.

RELATED APPEALS AND INTERFERENCES

The Appellants appealed to the PTO's Board of Patent Appeals and Interferences (BPAI) to contest a final rejection under 35 USC 112, first paragraph in U.S. Serial No. 08/260,190 (filed June 15, 1994) from which the instant application does not claim priority but is related. [U.S. Serial No. 08/260,190 also concerns the MN gene and protein, and contains claims to MN-related inventions by the same Appellants.] The BPAI's decision in that appeal concerning U.S. Serial No. 08/260,190 was in the Appellants' favor, reversing the 35 USC 112, first paragraph final rejection. A copy of that decision from the BPAI is attached in the RELATED PROCEEDINGS APPENDIX.

STATUS OF THE CLAIMS

Claims 1-30, 38, 40, 43 and 44 were previously cancelled. Claims 31-37, 39, 41 and 42, the only claims pending, are rejected in a Final Office Action mailed from the PTO on October 13, 2006, under two 35 USC 112, first paragraph rejections, from which this appeal is taken. The claims are identified in attached CLAIMS APPENDIX.

STATUS OF AMENDMENTS

No amendments have been filed since the October 13, 2006 Final Office Action from which this appeal is taken.

SUMMARY OF CLAIMED SUBJECT MATTER

There is one independent claim under appeal, Claim 31, from which Claims 32-37, 39, 41 and 42 depend. Appellants are not arguing separately concerning any of the dependent claims.

The Appellants, Zavada et al., discovered a new gene and protein called initially MN. Appellants found that the MN gene is present in the chromosomal DNA of all vertebrates tested, and that its expression is strongly correlated with tumorigenicity. [Specification, page 1, lines 16-18.] The MN gene is "a cellular gene considered to be an oncogene, which encodes the oncoprotein now known alternatively as the MN protein, the MN/CA IX isoenzyme or the MN/G250 protein." [Specification, page 1, lines 7-9.]

> MN/CA IX is a transmembrane protein located at the cell surface. . . .
>
> . . . .
>
> . . . The MN protein has an extracellular domain [amino acids (aa) 38-414 of Figure 1A-1C (SEQ ID NO: 87)], a transmembrane domain [aa 415-434 (SEQ ID NO: 52)] and an intracellular domain [aa 435-459 (SEQ ID NO: 53)]. The extracellular domain contains the **proteoglycan-like domain** [aa 53-111 (SEQ ID NO: 50)] and the carbonic anhydrase (CA) domain [aa 135-391 (SEQ ID NO: 51].

[Specification, page 18, line 32 to page 19, line 20; emphasis added.]

The claims under appeal concern methods of identifying molecules that bind to the cell adhesion site on the MN protein. That site was identified as explained in Example 2 entitled "Identification of MN's Binding Site" at page 62, line 9 to page 70, line 14 "**to be closely related or identical to the epitope for MAb M75** [MN-specific monoclonal antibody (the hybridoma for which is deposited at the ATCC)], at least two

copies of which are located in the 6-fold tandem repeat of 6 amino acids [aa 61-96 (SEQ ID NO: 97)] in the **proteoglycan-like domain** of the MN protein." [Specification, page 62, lines 29-32; emphasis added.]

The claimed methods concern cell adhesion assays wherein molecules are screened to determine if they inhibit the adhesion of vertebrate cells to MN's cell adhesion site identified as indicated above "to be closely related or identical to the epitope for MAb M75 . . . in the proteoglycan-like domain of the MN protein." [Specification, page 62, lines 29-32.] In independent Claim 31, the cell adhesion site "comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 98-103 . . ." wherein said SEQ ID NOS. 10 and 98-103 are each considered depending on the context to represent the epitope for the M75 MAb, to which epitope MN's cell adhesion site is considered "to be closely related or identical. . . ." [Specification, page 62, lines 29-30.]

Support for the identity of SEQ ID NOS. 10 and 98-103 representing the M75 MAb's epitope can be found in the Specification at least at page 50, lines 16-24 as quoted below:

> The M75 epitope is considered to be present in at least two copies within the 6X tandem repeat of 6 amino acids [aa 61-96 (SEQ ID NO: 97)] in the proteglycan domain of the MN protein. Exemplary peptides representing that epitope depending on the context may include the following peptides from that tandem repeat: EEDLPS (**SEQ ID NO: 10**; aa 62-67); GEEDLP (**SEQ ID NO: 98**; aa 61-66; aa 79-84; aa 85-90; aa 91-96); EEDL (**SEQ ID NO: 99**; aa 62-65; aa 80-83; aa 86-89; aa 92-95); EEDLP (**SEQ ID NO. 100**; aa 62-66; aa 80-84; aa 86-90; aa 92-96); EDLPSE (**SEQ ID NO: 101**; aa 63-68); EEDLPSE (**SEQ ID NO: 102**; aa 62-68); and DLPGEE (**SEQ ID NO: 103**; aa 82-87, aa 88-93).

[Emphasis added.]

The MN protein/polypeptide comprising said cell adhesion site used in the claimed methods is further specified in independent Claim 31 as being "specifically bound by the M75 monoclonal antibody that is secreted from the hybridoma VU-M75, which was deposited at the American Type Culture Collection under ATCC No. HB 11128 . . .", and still further by the encoding nucleic acid – MN's cDNA SEQ ID NO: 1 [Specification, Figure 1, page 17, lines 12-13], and sequences that hybridize to the complement of SEQ ID NO: 1 under stringent hybridization conditions, and nucleotide sequences that differ from SEQ ID NO: 1 or from the nucleotide sequences that hybridize to SEQ ID NO: 1 under stringent hybridization conditions in codon sequence due to the degeneracy of the genetic code.

The ATCC deposit information for the hybridoma VU-M75 that secretes the M75 MAb can be found in the Specification at least at page 2, lines 17-18, at page 8, lines 8-16, and at page 74, lines 15-16. The language used to describe the nucleic acids encoding the MN protein/polypeptide used in the claimed methods can be found at least at page 23, lines 10-16. [Analogous language concerning MN cDNA has been used in earlier Zavada et al. U.S. patents, for example, in U.S. Patent Nos. 5,387,676 and 6,027,887 wherein MN proteins and MN polypeptides are similarly described.]

The proviso at the end of independent Claim 31, which proviso is the subject of the second final 35 USC 112, first paragraph rejection from which this appeal is taken, was added to make explicit what one of skill in the art would understand from the implicit teachings of the Specification. That proviso points out that if an MN fusion protein/polypeptide is used in the claimed methods, that "the non-MN portion of said fusion protein or said fusion polypeptide . . . [cannot] contain a cell adhesion site" for the

claimed methods to be operative. Support for that proviso can be found at least at page 21, lines 1-14, wherein the use of fusion proteins in the cell adhesion assays is described; and at page 69, lines 8-13, wherein the lack of utility of the fusion protein GST-MN in the cell adhesion assay is attributed to the presence of a second cell adhesion site in the non-MN (GST) portion of the fusion protein.

The Specification reads at page 69, lines 8-13:

> There can be no doubt on the specificity of cell attachment to purified MN/CA IX+. It is abrogated by specific MAb M75, at a dilution 1:1000 of ascites fluid. This is a correction to our previous report in Zavada et al., Int. J. Oncol., 10: 857 (1997) in which we observed that MN/CA IX produced by vaccinia virus vector and fusion protein GST-MN support cell adhesion, but we did not realize that GST anchor itself contains another binding site, which is not blocked by M75.

That passage at page 69, lines 8-13 of the Specification teaches that the use of the MN fusion protein GST-MN in previous experiments had masked the identification of MN's cell adhesion site and had led to incorrect conclusions, because the inventors had not realized that the "GST [glutathione-S-transferase] anchor itself contains another binding site, which is not blocked by M75." [Specification at page 69, lines 12-13. M75 is a monoclonal antibody that specifically binds MN protein at its cell adhesion site.] The GST-MN fusion protein is then expressly described in the Specification as an inoperative embodiment that renders useless a cell adhesion assay to detect molecules that bind MN's cell adhesion site, in that the MN fusion protein's non-MN portion "itself contains another binding site, which is not blocked by M75." [*Id.*]

That concept of the extra binding site rendering the assay inoperative is graphically illustrated in the attached Evidence Appendix 1. [The Examiner had

suggested that using different vertebrate cells in the methods, that is HeLa cells rather than NIH3T3 cells as used in Zavada et al. (1997), would have changed the result. However, as illustrated in Evidence Appendix 1, it is the second cell binding site in the non-MN portion of the fusion protein that is the problem creating inoperability, not the type of vertebrate cells used.]

Ones of skill in the art would then understand from the Specification that any non-MN portion of a MN fusion protein/polypeptide used in the claimed cell adhesion assays could not contain its own cell adhesion site for the cell adhesion assay to be effective to detect molecules that bind to MN's cell adhesion site. Ones of skill in the art could predict from the Specification [particularly at page 69, lines 8-13] that, if the MN fusion protein GST-MN did not work in the claimed assay because the GST portion of it contained its own binding site, then any other MN fusion protein containing a second, non-MN cell adhesion site would also not work in the claimed assay. The proviso at issue simply expresses that understanding.

The Specification teaches that if a MN fusion protein/polypeptide is used in a MN cell adhesion assay, the non-MN portion of the fusion protein needs to be tested to assure that it does not contain a cell binding site. By the addition of the proviso to the end of Claim 31, Appellants only made explicit what one of skill in the art would understand from the implicit teachings of the Specification: that an MN fusion protein containing a second, non-MN protein-derived cell adhesion site would not be useful to screen for molecules that bind to MN's cell adhesion site.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The subject claims, Claims 31-37, 39, 41 and 42, have been finally rejected under two 35 USC, first paragraph rejections.

First 35 USC 112, First Paragraph Rejection

Claims 31-37, 39, 41 and 42 stand rejected under 35 USC, first paragraph

> because the claimed "amino acid sequence" of the site still reads on a sequence as short as two amino acids derived from any of the claimed sequences listed in the Markush group. In other words, limiting the sequence by using Markush type language does not satisfy the lack of written description for a sequence of anything other than the sequence consisting of any one of SEQ ID NO: 10, 98-102 or 103. Again it is reiterated that Appellant may overcome this rejection by amending the claims to indicate "the" sequence as opposed to "a sequence".

[Final Office Action mailed from the PTO on October 13, 2006, paragraph bridging pages 3-4.] The Examiner in that rejection is referring to lines 5-8 of independent Claim 31. The Examiner in referring to "'the' sequence" as opposed to "a sequence" is referring to the phrase "**an** amino acid **sequence** selected from the group . . ." at lines 5-6 of independent Claim 31.

Second 35 USC 112, First Paragraph Rejection "New Matter Rejection"

Claims 31-37, 39, 41 and 42 stand rejected under 35 USC 112, first paragraph because independent Claim 31 was amended to contain at its end the above-identified negative proviso. The October 13, 2006 Final Office Action states at page 3:

> The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. THIS IS A NEW MATTER REJECTION.
>
> Appellant's have amended the claims to include a negative proviso limitation of "the non-MN-portion of said fusion protein or said fusion polypeptide does not contain a cell adhesion site". Appellant directs the examiner to page 21, lines 1-14 and page 69, lines 8-13 for support of this new limitation. However, the pages direct are drawn to the explanation of why the fusion protein would contain an additional binding site to which cells could potentially bind. There is not specific indication or disclosure that support a negative limitation or specific exclusion of fusion proteins missing a cell adhesion site as now currently claimed.

[Emphasis in original.]

ARGUMENT

First 35 USC §112, ¶1 Rejection is Improper: Use of Formal Markush Group Language

Appellants respectfully submit that the first 35 USC §112, ¶1 rejection of the October 13, 2006 Final Office Action is improper as it constitutes a rejection of standard Markush group claim language in independent Claim 31. Appellants respectfully submit that the phrase at issue from the first paragraph of Claim 31 -- "wherein said site's amino acid sequence comprises **an** amino acid sequence selected from the group consisting of SEQ ID NOS.: 10 and 98-103 . . ." (emphasis added) -- is formal Markush group claim language that inherently refers to the full-length amino acid sequence of one of a group of amino acid sequences identified by SEQ ID NOS., and would **not** be understood by ones of skill in the art to include within said group "a fragment as small as two amino acids . . ." from within one of the listed amino acid sequences, as argued by the Examiner in the April 24, 2006 Office Action at page 7, and in the October 13, 2006 Final Office Action at pages 2-3.

Appellants respectfully submit that they would have amended Claim 31 to read "wherein said site's amino acid sequence comprises [[an]] **the** amino acid sequence selected from SEQ ID NOS: 10 and 98-103 . . ." as suggested by the Examiner, but were concerned that said "the" would render the "amino acid sequence" it modifies with an ambiguous antecedent. Would that "amino acid sequence" modified by "the" be "said site's amino acid sequence," when "said site's amino acid sequence comprises" said amino acid sequence?[1]

---

1. As indicated in the above Summary of Claimed Subject Matter, the Specification discloses in Example 2 entitled "Identification of MN's Binding Site" that "the

Therefore, instead Appellants amended Claim 31 to address the 112, first paragraph rejection to read in its first paragraph that "said site's amino acid sequence comprises an amino acid sequence selected from **the group consisting of** SEQ ID NOS: 10 and 98-103. . . ." Appellants respectfully submit that that formal Markush group language addresses the Examiner's concern that "a sequence as short as two amino acids . . ." [October 13, 2006 Final Office Action, page 2] from within the listed amino acid sequences could be meant.[2] Appellants have and do remain respectfully open to alternative claim language with the equivalent intended meaning.

Appellants respectfully conclude that the invention as disclosed in the Specification as identified above was well within the Appellants' possession at the time the instant application was filed. Appellants respectfully request that the BPAI reverse

---

binding site of MN was determined to be closely related or identical to the epitope for MAb M75. . . ." [Specification, page 62, lines 29-30.] The Specification further points out at least at page 50, lines 18-24, that "[e]xemplary peptides representing . . . [the M75 MAb] epitope" include SEQ ID NOS: 10 and 98-103. Then the Specification teaches that MN's cell adhesion site is not "the amino acid sequence" of any of the exemplary peptides representing the M75 MAb epitope, but could comprise one of those exemplary peptides of SEQ ID NOS: 10 and 98-103.

2. Appellants respectfully submit that the standard Markush claim language of Claim 31 (amended or unamended) inherently refers to the **full-length amino acid sequence** of one of a group of SEQ ID NOS rather than to amino acid sequences of fragments derived from the SEQ ID NOS. For example, using the search function for patent terms on the USPTO website [http://patft.uspto.gov/netahtml/PTO/search-bool.html], a total of 2,217 patents were found with claims that use that phrase recited in unamended Claim 31, but only **one** patent found that qualifies the amino acid sequence as "full-length" (i.e., "a **full-length** amino acid sequence selected from. . . ."; see Claim 1 of US Patent No. 6,852,832). However, for greater clarity and particularity, the pertinent phrase in Claim 31 of the instant application has been amended to read, "amino acid sequence selected from **the group consisting of**. . . .". According to the USPTO website, that phrase has been allowed in the claims of 2,055 patents.

the instant 35 USC 112, first paragraph rejection in view of the above remarks and the standard Markush group language at issue.

Second 35 USC §112, ¶1 Rejection is Improper: Rejection of Inherent Characterization as New Matter

Appellants respectfully submit that the second 35 USC §112, ¶1 rejection of the October 13, 2006 Final Office Action is improper as a "new matter" rejection of subject matter that is not "new matter" but instead constitutes a negative limitation clearly inherent in the Specification. Appellants respectfully point out that it is axiomatic that the clarification of inherent characterization does not add new matter to an application. [See, for example, In re Smythe, 178 USPQ 279 (CCPA 1973).]

The instant 35 USC §112, ¶1 rejection is based on a finding of lack of literal support in the Specification for a proviso added to the end of Claim 31, and perhaps on a misunderstanding:

> Applicant's [sic] have amended the claims to include a negative proviso limitation of "the non-MN-portion of said fusion protein or said fusion polypeptide does not contain a cell adhesion site". Applicant directs the examiner to page 21, lines 1-14 and page 69, lines 8-13 for support of this new limitation. However, the pages direct [sic] are drawn to the explanation of why the fusion protein would contain an additional binding site to which cells could potentially bind. There is no specific indication or disclosure that support a negative limitation or specific exclusion of fusion proteins missing a cell adhesion site as now currently claimed.

[October 13, 2006 Final Office Action, page 3, Section 5.] Appellants respectfully respond that the addition of the proviso at the end of Claim 31 only makes explicit what one of skill in the art would understand from the implicit teachings of the Specification.[3]

The MPEP at §2163.07(a) makes clear that a specification is interpreted according to what one of ordinary skill in the art would understand is supported both explicitly and implicitly, and that the claims may be amended accordingly without adding new matter:

> By disclosing in a patent application a device that inherently performs a function, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing the prohibited new matter. *In re Reynolds* . . . 170 USPQ 94 (CCPA 1971); *In re Smythe* . . . [cited supra] (CCPA 1973).

As the PTO Board of Patent Appeals and Interferences stated in Ex parte Soreson, 3 USPQ2d 1462 (Bd. Pat. App. & Interf. 1987) at page 1463:

> [W]e are mindful that appellant's specification need not describe the claimed invention in *ipsis verbis* to comply with the written description requirement. . . . The test is whether the originally filed specification disclosure *reasonably* conveys to a person having ordinary skill that applicant had possession of the subject matter later claimed. . . .

[Emphasis in original.]

Regarding negative limitations, the MPEP at §2173.05(i) requires only that

> [a]ny negative limitation or exclusionary proviso must have basis in the original disclosure. . . . [A] lack of literal basis in the specification for a negative limitation may not be

---

3. "To comply with the written description requirement of 35 USC 112, para. 1, . . . each claim limitation must be expressly, implicitly, or inherently supported in the originally filed disclosure." [MPEP §2163.05, page 181.]

> sufficient to establish a *prima facie* case for lack of descriptive support. *Ex parte Parks*, 30 USPQ2d 1234, 1236 (Bd. Pat. App. & Inter. 1993).

There is a clear basis in the original Specification for the proviso at issue. The passage at page 69, lines 8-13 of the Specification, quoted by Appellants above as support for the proviso of Claim 31, teaches that the use of the MN fusion protein GST-MN in previous experiments had masked the identification of MN's cell adhesion site and had led to incorrect conclusions, because the inventors had not realized that the "GST [glutathione-S-transferase] anchor itself contains another binding site, which is not blocked by M75." [Specification at page 69, lines 12-13. M75 is a monoclonal antibody that specifically binds MN protein at its cell adhesion site.] The GST-MN fusion protein is then expressly described in the Specification as an inoperative embodiment that renders useless a cell adhesion assay to detect molecules that bind MN's cell adhesion site, in that the MN fusion protein's non-MN portion "itself contains another binding site, which is not blocked by M75." [*Id.*]

As explained above in the Summary of Claimed Subject Matter, Evidence Appendix 1 graphically illustrates the concept made implicit in the negative proviso ending independent Claim 31. One of skill in the art would understand from the Specification that whereas Zavada et al. (1997) [copy attached as Evidence Appendix 2] taught away from the epitope for the M75 MAb being "closely related or identical" to MN's cell adhesion site, that the instant application corrected Zavada et al. (1997), and in doing so not only taught the identity of MN's cell adhesion site, but also taught that one of skill in the art must beware of a MN fusion protein/polypeptide that contains a cell adhesion site in its non-MN portion for use in the claimed methods.

Ones of skill in the art would then understand from the Specification that any non-MN portion of a MN fusion protein/polypeptide used in the claimed cell adhesion assays could not contain its own cell adhesion site for the cell adhesion assay to be effective to detect molecules that bind to MN's cell adhesion site. Ones of skill in the art could predict from the Specification [particularly at page 69, lines 8-13] that, if the MN fusion protein GST-MN did not work in the claimed assay because the GST portion of it contained its own binding site, then any other MN fusion protein containing a second, non-MN cell adhesion site would also not work in the claimed assay. The proviso at issue simply expresses that understanding.

The Specification teaches that if a MN fusion protein/polypeptide is used in a MN cell adhesion assay, the non-MN portion of the fusion protein needs to be tested to assure that it does not contain a cell binding site. By the addition of the proviso to the end of Claim 31, Appellants are only making explicit what one of skill in the art would understand from the implicit teachings of the Specification: that an MN fusion protein containing a second, non-MN protein-derived cell adhesion site would not be useful to screen for molecules that bind to MN's cell adhesion site.

Misconceptions concerning the identity of MN's binding site in Zavada et al., Int. J. Oncol., 10: 857 (1997) [cited herein as "Zavada et al. (1997)"; copy attached in Evidence Appendix 2] arose from the nature of the MN-GST fusion protein used. The Specification corrects the misconceptions concerning the identity of MN's binding site in Zavada et al. (1997). For example, the Specification at page 69, lines 8-13 reads:

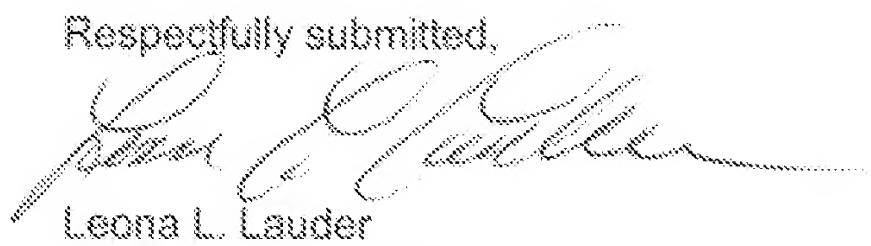
> There can be no doubt on the specificity of cell attachment to purified MN/CA IX+. It is abrogated by specific MAb M75, at a dilution 1:1000 of ascites fluid. **This is a correction to our previous report in Zavada et al.,**

> Int. J. Oncol., 10: 857 (1997) in which we observed that MN/CA IX produced by vaccinia virus vector and fusion protein GST-MN support cell adhesion, but **we did not realize that GST anchor itself contains another binding site, which is not blocked by M75**.

[Emphasis added.] The Specification teaches that if a MN fusion protein is used in a cell adhesion assay, that the non-MN portion of the fusion protein needs to be tested to assure that it does not contain a cell binding site, or that if the non-MN portion of the fusion protein contains a cell binding site, that said site must be blocked. Otherwise, the assay, as that of Zavada et al. (1997), would not have utility.

Appellants respectfully conclude that no new matter was added by the addition of the negative proviso at the end of Claim 31 which renders what was implicit in the Specification implicit in independent Claim 31. For the reasons provided above, Appellants respectfully request that the second 35 USC §112, ¶1 rejection of all the pending claims be reversed.

Respectfully submitted,



Leona L. Lauder
Attorney for Appellants
Registration No. 30,863

Dated: February 22, 2007

CLAIMS APPENDIX

Claim 31: A method of identifying an organic or an inorganic molecule that binds specifically to MN's cell adhesion site, to which site vertebrate cells adhere in a cell adhesion assay, wherein said site is within MN's proteoglycan-like domain, wherein said site's amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 98-103, said method comprising testing an organic or an inorganic molecule in a cell adhesion assay, wherein said cell adhesion assay comprises:

(a) allowing MN protein, which comprises said site, and/or MN polypeptide, which comprises said site, to bind to a substrate, to which substrate vertebrate cells do not bind;

(b) rinsing unbound MN protein or unbound MN polypeptide from said substrate;

(c) incubating the bound MN protein or the bound MN polypeptide with said organic or inorganic molecule, and with said vertebrate cells;

(d) rinsing unbound vertebrate cells from said bound MN protein or bound MN polypeptide; and

(e) if said organic or said inorganic molecule inhibits the adhesion of said vertebrate cells to said MN protein or to said MN polypeptide, identifying said molecule as specifically binding to said site;

wherein said site, and said MN protein or said MN polypeptide, are specifically bound by the M75 monoclonal antibody that is secreted from the hybridoma

VU-M75, which was deposited at the American Type Culture Collection under ATCC No. HB 11128, and wherein said MN protein or said MN polypeptide is encoded by a nucleotide sequence selected from the group consisting of:

(i) SEQ ID NO: 1;

(ii) nucleotide sequences that hybridize specifically under stringent hybridization conditions of 0.02 M to 0.15 M NaCl at temperatures of 50°C to 70°C to the complement of SEQ ID NO: 1; and

(iii) nucleotide sequences that differ from SEQ ID NO: 1 or from the nucleotide sequences of (ii) in codon sequence due to the degeneracy of the genetic code;

and wherein if said MN protein or said MN polypeptide is a fusion protein or a fusion polypeptide, the non-MN portion of said fusion protein or said fusion polypeptide does not contain a cell adhesion site.

Claim 32: The method of Claim 31 wherein said molecule is organic.

Claim 33: The method of Claim 31 wherein said molecule is inorganic.

Claim 34: The method of Claim 32 wherein said molecule is a protein or a polypeptide.

Claim 35: The method of Claim 34 wherein said protein or polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 137 and 138.

Claim 36: The method of Claim 34 wherein said polypeptide is selected from the group consisting of SEQ ID NOS: 137 and 138.

Claim 37: The method of Claim 31 wherein said organic or inorganic molecule, when in contact with a vertebrate preneoplastic or neoplastic cell that abnormally expresses MN protein, inhibits the growth of said cell.

Claim 39: The method of Claim 31 wherein said MN polypeptide is SEQ ID NO: 106.

Claim 41: The method of Claim 31 wherein said vertebrate cells are mammalian cells.

Claim 42: The method of Claim 31 wherein said vertebrate cells are human cells.

EVIDENCE APPENDICES 1 AND 2

The attached Evidence Appendix 1 was submitted as APPENDIX A at the end of the AMENDMENT AND REQUEST FOR CONTINUED EXAMINATION (RCE) submitted to the PTO by the Appellants on February 2, 2006, and had been submitted to the Examiner on November 3, 2005 for purposes of a telephone interview on November 7, 2005. The Appendix was included in an Interview Summary prepared by the Examiner and mailed from the PTO on November 17, 2005.

The Evidence Appendix 2 is a copy of Zavada et al., International Journal of Oncology, 10: 857-863 (1997) [herein cited as "Zavada et al. (1997)"] upon which the Examiner relied in 35 USC 102(b) rejections in Office Actions mailed from the PTO on April 24, 2006, September 9, 2005, February 9, 2005, and February 12, 2003, and in 103(a) rejections in the Office Actions mailed from the PTO on April 24, 2006, September 9, 2005, and February 9, 2005.

Appellants respectfully point out that said 35 USC 102(b) and 103(a) rejections are not present in the Final Office Action mailed from the PTO on October 13, 2006, from which the instant appeal is made. However, the October 13, 2006 Final Office Action states on page 4 [at the end of a section entitled "NEW REJECTION" which is a "NEW MATTER REJECTION"]:

> It is noted that the art rejections of record do not anticipate the claims as currently presented. However, if Appellant reverts to the claims previously presented, wherein there is an exclusion of the negative limitation of no [sic] containing a cell adhesion site, the art rejection may be re-applied.

Evidence Appendix 1

Zavada et al., Int. J. Oncology, 10: 857-863 (1997)

If HeLa cells were substituted for NIH3T3 cells in the cell adhesion assay of Zavada et al., Int. J. Oncology, 10: 857-863 (1997), the M75 monoclonal antibody would still not have blocked the binding of vertebrate (HeLa) cells to the GST-MN fusion protein.

In the diagram, (A) shows the result of M75 Mab incubation with the GST-MN fusion protein before adding NIH3T3 cells (as in Zavada et al. 1997): the NIH3T3 cells appear to not be blocked from binding to the MN cell adhesion site because they bind to a second cell adhesion site located in the GST anchor.[1]

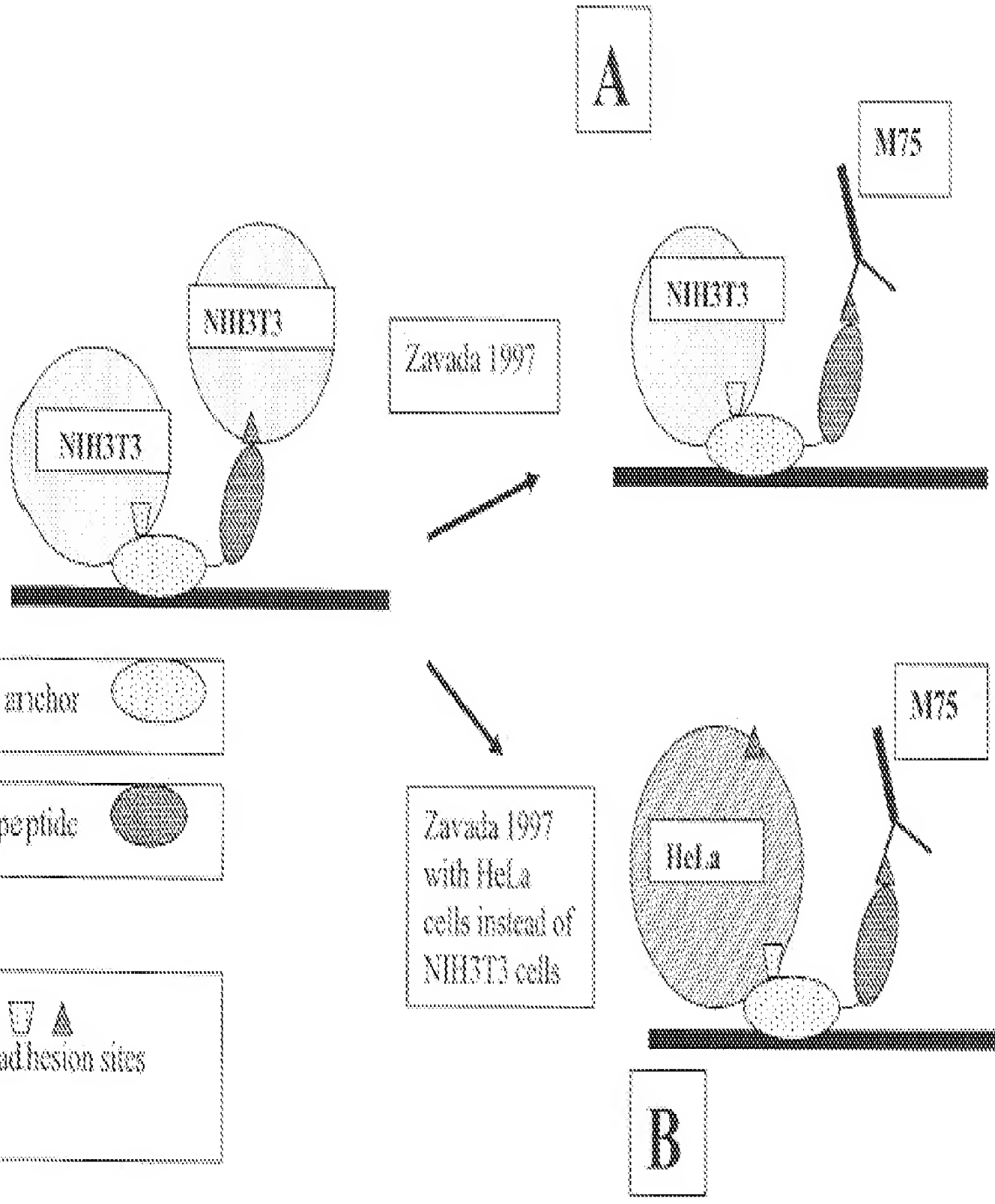
In (B), if MN-positive human HeLa cells are substituted for NIH3T3 cells in the experiment described in Zavada et al. 1997, they would also appear to not be blocked from binding to the MN cell adhesion site because the HeLa cells (although blocked from binding to the MN cell adhesion site on the MN part of the fusion protein) would also bind to the GST cell adhesion site.

---

1 The instant application at page 69, lines 8-13 corrects the error in the 1997 Zavada et al. article by stating:

> There can be no doubt on the specificity of cell attachment to purified MN/CA IX+. It is abrogated by specific MAb M75. . . . **This is a correction to our previous report in Zavada et al., Int. J. Oncol., 10: 857 (1997) in which we observed that MN/CA IX produced by vaccinia virus vector and fusion protein GST-MN support cell adhesion, but we did not realise that GST anchor itself contains another binding site, which is not blocked by M75.**

[Emphasis added.]


A
M75
NIH3T3
NIH3T3
Zavada 1997
NIH3T3
GST anchor
MN peptide
Cell adhesion sites
Zavada 1997
with HeLa
cells instead of
NIH3T3 cells
M75
HeLa
B

INTERNATIONAL JOURNAL OF ONCOLOGY 10: 697-703, 1997

Transient transformation of mammalian cells by MN protein, a tumor-associated cell adhesion molecule with carbonic anhydrase activity

JAN ZAVADA[1], ZUZANA ZAVADOVA[1], ONDREJ MACHON[1], LUDA KUTINOVA[2], SARKA NEMECKOVA[2], RENE OPAVSKY[3] and JAROMIR PASTOREK[3]

[1]Institute of Molecular Genetics, Academy of Sciences of Czech Republic; [2]Institute of Hematology and Blood Transfusion, Prague, Czech Republic; [3]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Received January 30, 1997; Accepted February 14, 1997

Abstract. The MN protein is associated with certain human carcinomas, but absent in most normal tissues. It is a transmembrane protein; its extracellular part contains a domain homologous with carbonic anhydrases (CAs) and a proteoglycan-like region. In the present study, we observed that cells (human CGL1 and mouse NIH3T3 cells) transfected with MN cDNA showed morphologic transformation, but reverted to normal phenotype after 4-5 weeks. This reversion was not due to the loss, silencing, or mutations of MN insert. We also found that MN protein exerted CA enzymatic activity, but this was not relevant for morphologic transformation of cells. MN is an adhesion protein, involved in cell-to-cell contacts, this probably could explain its role in tumorigenesis.

Introduction

The MN protein was first discovered by monoclonal antibody M75 in HeLa cells and in several types of human carcinomas (cervical, ovarian, endometrial). It is absent in normal human fibroblasts and in normal organs from which these tumors originate. It is a 'twin' protein of 54 and 58 kDa, which forms oligomers of 153 kDa linked with disulfidic bonds (1,2).

A unique feature of MN protein, as revealed by immunohistological examination, is its very high association with cervical carcinomas and precancероses, and its absence in all normal tissues except for alimentary tract mucosa (3-7).

Sequence of MN-cDNA and gene has been determined (8,9). According to the predicted amino acid sequence, MN protein (Fig. 1) consists of a signal peptide, a proteoglycan-like region, a large domain homologous to CAs (with a conserved enzyme active site, including a zinc-binding structure), of a transmembrane segment and a short intracellular domain. MN-cDNA, inserted into an eukaryotic expression vector, induced morphologic transformation of transfected mouse NIH3T3 cells (8).

The present work brings new data concerning transformation of mammalian cells by MN-cDNA inserted into expression vectors derived from retroviruses. Cells transfected with these constructs showed morphologic transformation, but after some time, they reverted to normal phenotype. We have tried to explain the mechanism of reversion.

Sulfonamides, including acetazolamide, are very potent inhibitors of known CAs (10). Acetazolamide was tested to determine if it inhibited also MN-CA and if so, whether inhibition of the enzyme affected cell transformation.

There are reasons to believe that MN protein could be involved in direct cell-to-cell interactions: i) Previous observations indicated a functional resemblance of MN protein to surface glycoproteins of enveloped viruses, which mediate virus adsorption to cell surface receptors, and MN participated in formation of phenotypically mixed virions of vesicular stomatitis virus. In the beginning, we even thought that MN could be a viral protein (1,2). ii) Inducibility of MN protein expression by growing HeLa cells in densely packed monolayers suggests that it may be involved in direct interactions between the cells (1). iii) Finally, there is a structural similarity between the MN protein (Fig. 1) and receptor tyrosine phosphatase β, which also contains proteoglycan and CA domains; those domains mediate direct contacts between cells of the developing nervous system (11). Therefore, we tested if MN protein binds to cell surface receptors; the result clearly showed that it does.

Materials and methods

Cell lines. Cells used in the present work were: HeLa x fibroblast hybrids (non-tumorigenic CGL1 and tumorigenic

Correspondence to: Dr Jan Zavada, Institute of Molecular Genetics, Flemingovo nam. 2, 166 37 Prague 6, Czech Republic

Abbreviations: BSA, bovine serum albumin; CA, carbonic anhydrase; CAM, cell adhesion molecule; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; PBS, phosphate buffered saline; SAC, Staphylococcus aureus cells

Key words: oncogene, cell adhesion molecule, carbonic anhydrase, tumor suppressor gene

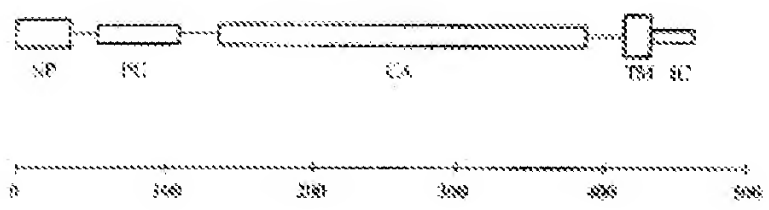


Figure 1. Structure of MN protein. SP, signal peptide; PG, proteoglycan like region; CA, carbonic anhydrase domain; TM, transmembrane anchor; IC, intracellular tail. The scale shows No. of amino acid residues. The scheme is based on refs. 8 and 9, EMBL Data Library under the accession number X66839, updated 12/08/95.

CGL3) (12); mouse cell line NIH3T3, subclone 2; HeLa cells and monkey Vero cells (1,2).

Expression vectors. Full-length MN cDNA was acquired from pBluescript subclone (8). To get rid of 5 and 3-end noncoding sequences, that might reduce subsequent gene expression, a polymerase chain reaction (PCR) was performed. 5' primer TAGACAGATCTACGATGGCTCCC CTGTGCCCCAG encompasses translation start site and BglII cloning site and 3' primer ATTCCTCTAGACAGTTA CCGGCTCCCCCTCAGAT encompasses stop codon and XbaI cloning site. Full-length MN-cDNA as a template and Pfu DNA polymerase (Stratagene) were used in the reaction. The PCR product was sequenced and found identical with template; it carried no mutations. The PCR product harbouring solely MN coding sequence was inserted into three vectors: a) pMAMneo (Clontech) plasmid allowing dexamethasone-inducible expression driven by the MMTV-long terminal repeat (LTR) promoter and containing *neo* gene for selection of transformants in media supplemented with Geneticin (G418) antibiotic. b) Retroviral expression vector pGD (13) containing MLV-LTR promoter and *neo* gene for G418 antibiotic selection. c) Vaccinia virus expression vector pSC11 (14). Transfection was performed via calcium-phosphate precipitate according to ref. 15.

Vaccinia virus strain Praha clone 13 was used as parental virus (16). Vaccinia virus recombinant was prepared by standard procedure (17). Recombinant viruses were selected and plaque purified twice in rat thymidine-kinase-less RAT2 cells (18) in the presence of 5-bromodeoxyuridine (100 μg/ml). Blue plaques were identified by overlaying with agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (200 μg/ml).

CA assay. CA activity was measured by a micro-method (19). In principle, velocity of the reaction $CO_2 + H_2O \leftrightarrow H_2CO_3$ is measured by the time required for acidification of carbonate buffer in CO_2 atmosphere, detected with phenol red as a pH indicator. This reaction proceeds even in absence of the enzyme, with t_0 = control time (this was set to 60 sec). Carbonic anhydrase reduces the time of acidification to t; one unit of the enzyme activity reduces the time to one half of control time: $t/t_0 = 1/2$. On the plot used in Fig. 6 this corresponds to $t_0/t-1 = 1$. For the experiment MN protein was immunoprecipitated with Mab M75 from RIPA buffer (1% Triton X-100, 0.1% deoxycholate, 1 mM phenylmethyl-

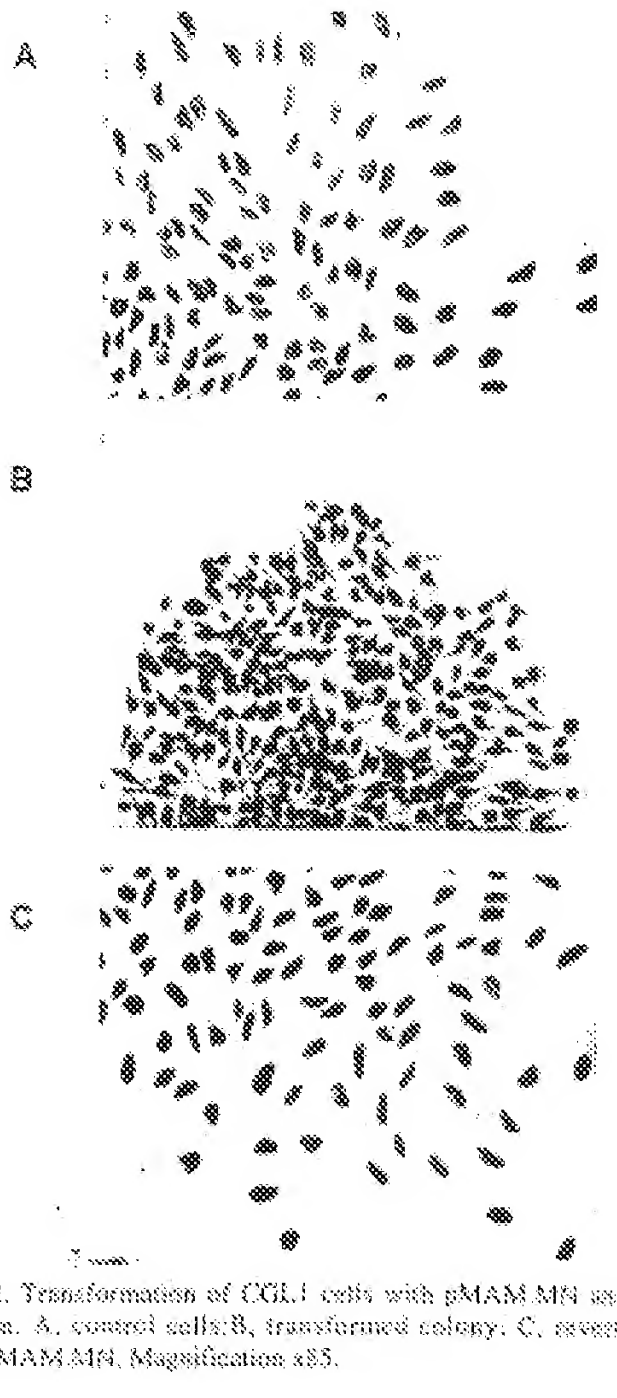


Figure 2. Transformation of CGL3 cells with pMAM.MN and following reversion. A, control cells; B, transformed colony; C, reverted colony CGL3/pMAM.MN. Magnification ×85.

sulfonylfluoride and 200 trypsin-inhibiting units/ml of Trasylol in PBS, pH 7.2) extract of Vero cells infected with vaccinia, MN construct, or with 'empty' vaccinia as a control. The MN+antibody complex was subsequently adsorbed to protein A-SAC (20) and rinsed 2x with PBS and 2x with 1 mM carbonate buffer, pH 8.0. The precipitate was resuspended in the same buffer and added to the reaction mixture. Acetazolamide (Sigma) was tested for inhibition of CA (10). In extracts of infected cells used for immunoprecipitation, the concentration of total proteins was determined by a previously described method (21) and that of MN protein by competition radioimmunoassay (2).

Western blots. Western blotting and development of the blots using ^{125}I-labelled M75 and autoradiography was performed as before (1,2).

Adhesion assay. For adhesion assay (22), 25 μl aliquots of MN protein (affinity purified pGEX-3X MN) (2) or of control proteins were spotted on 5 cm diameter bacteriological Petri dishes and allowed to bind for 2 h at room temperature. This yielded circular protein-coated areas of 4-5 mm diameter. MN protein was diluted to 10 μg/ml in 50 mM carbonate buffer, pH 9.2. Patches of adsorbed control proteins were prepared similarly. These included collagens type I and IV,

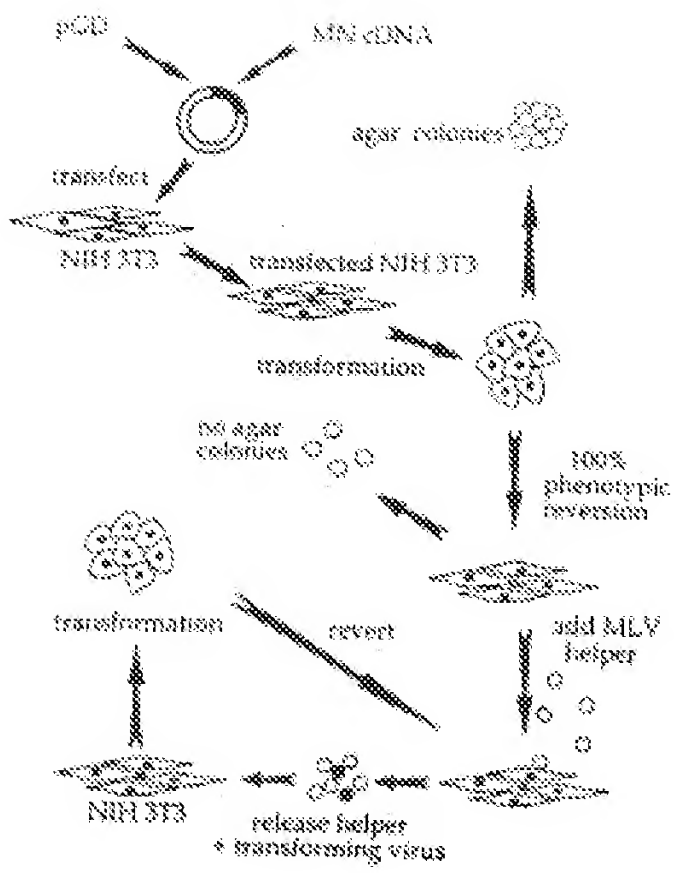


Figure 3. A scheme of experiment designed to explain the mechanism of reversion of NIH3T3 cells transformed with pGD.MN. For details see the text.

fibronectin, laminin and gelatin (Sigma), diluted and absorbed according to manufacturer's recommendations; FCS and BSA were also included. After aspiration of the drops, the dishes were rinsed 2x with PBS and saturated for 1 h with DMEM supplied with 5% FCS. The plates were seeded with $5x10^5$ cells in 5 ml of DMEM+5% FCS and incubated overnight at 37°C. The plates were rinsed with PBS and the cells attached were fixed with formaldehyde, post-fixed with methanol and Giemsa stained.

Results

Transformation and reversion of CGL1 cells transfected with MN-cDNA. Since the expression of MN protein correlated with tumorigenicity of HeLa x fibroblast hybrids (2), the non-tumorigenic hybrid CGL1 was first tested. Those cells, transfected with pMAM.MN construct, after selection with Geneticin formed colonies with varying degrees of transformation; some of them appeared normal. While normal CGL1 cells (Fig. 2A) are contact inhibited, growing in a parallel orientation, the transformed cells (Fig. 2B) formed very dense colonies, showing the loss of contact inhibition. Such colonies grew more slowly than original CGL1. After subcloning, the cells isolated from transformed colonies segregated revertants. The reversion was a gradual, step-wise process; there were colonies with different degree of reversion. After 2 passages (Fig. 2C), all the cell population became morphologically indistinguishable from normal CGL1. This was due to reversion of some cells and to selective advantage of the revertants, which grew faster than transformed cells. Despite repeated attempts, not even one single stably transformed cell clone was obtained. No transformed colonies were found in CGL1 cells transfected with 'empty' pMAM control plasmid.

Growth of the CGL1+pMAM.MN revertants in media supplied with 5 µg/ml of dexamethasone for 7 days enhanced the production of MN protein, but morphology of the cells did not return to transformed.

Rescue of transforming MN from the revertants. The reversion of MN-transformed cells to normal phenotype may have at least 5 causes: i) Loss of the MN insert; ii) Silencing of the MN insert, e.g., by methylation; iii) Mutation of the MN insert; iv) Activation of a suppressor gene, coding for a product which neutralizes transforming activity of MN protein; v) Loss of an MN-binding protein. To decide among these alternatives, the following experiment was designed (scheme in Fig. 3): MN-cDNA was inserted into pGD, a vector derived from mouse leukemia virus - MLV. A defective virus was thereby engineered, which contained the MN gene and the selective marker *neo* instead of genes coding for viral structural proteins. With this construct, mouse NIH3T3 cells were transfected. In media supplied with geneticin, the cells formed colonies with phenotype ranging from strongly transformed to apparently normal. All the transformed colonies and about 50% of normal colonies expressed MN protein. Contrasting with normal NIH3T3, the transformants were also able to form colonies in soft agar, reflective of the loss of anchorage dependence, characteristic for cell transformation. Upon passaging, the cells isolated from transformed colonies reverted to normal morphology, and at the same time, they lost the capacity to form colonies in soft agar, while still expressing the MN protein. This permanent presence of MN protein in revertants ruled out alternatives i) and ii), loss or silencing of MN gene as a cause of reversion.

To decide between the other 3 alternatives, we superinfected the revertants with live, replication competent MLV. This virus grows in NIH3T3 cells without morphologic manifestations, and it works as a 'helper' for the pGD.MN construct. Virus progeny from MLV-infected revertants represents an artificial virus complex [pGD.MN+MLV]. This consists of 2 types of virions: of standard type MLV particles and virions containing pGD.MN genome, enveloped in structural proteins provided by the 'helper' virus. This virus complex was infectious for fresh NIH3T3 cells; it again induced in them morphologic transformation and the capacity to form agar colonies.

Contrasting with NIH3T3 transfected with pGD.MN, all the colonies of cells infected with [pGD.MN+MLV] complex, which grew in the presence of geneticin, were uniformly transformed and contained MN protein.

The transformants once more reverted to normal phenotype although they kept producing the infectious [pGD.MN+MLV] complex, which induced the transformation in fresh NIH3T3 cells. This cycle of infection-transformation-reversion was repeated 3 times with the same result. This ruled out the alternative iii) - mutation of MN-cDNA as a cause of reversion.

This experiment is documented on Figs. 4 and 5, while leaving out unnecessary repeating of nearly identical photomicrographs at all the steps. Normal NIH3T3 cells (Fig. 4A) formed a contact inhibited monolayer of flat cells. Cells infected with [pGD.MN+MLV] complex were clearly transformed (Fig. 4B): they grew in a chaotic pattern and showed loss of contact inhibition. Some of the cells were

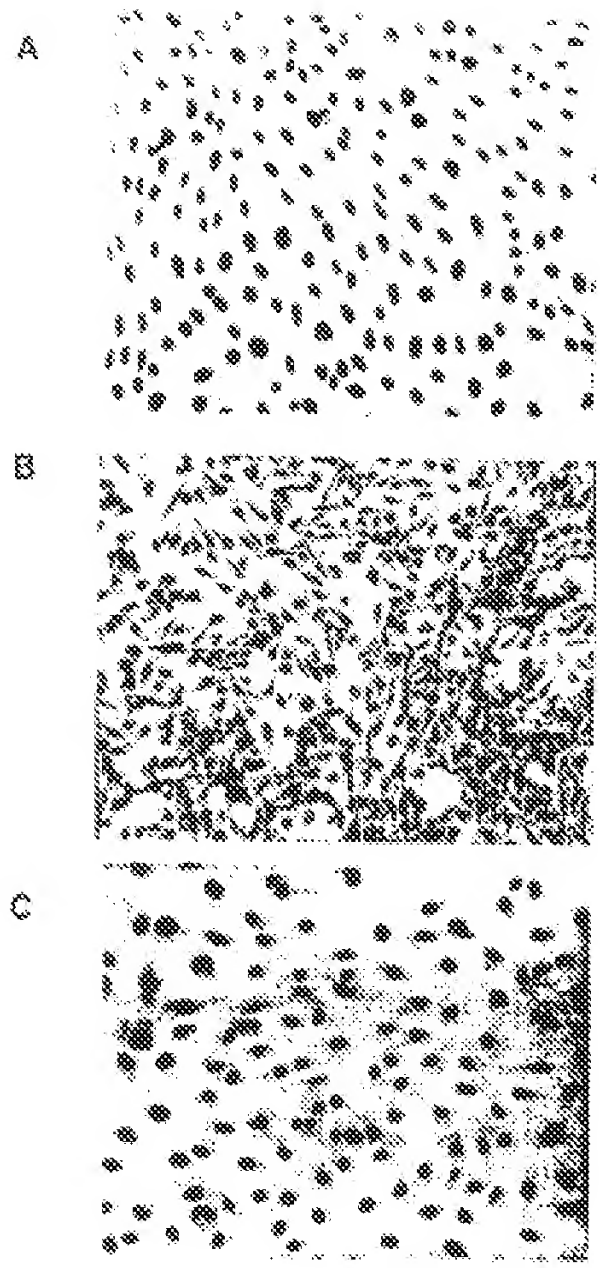


Figure 4. Transformation of NIH3T3 cells with [pGD.MN+MLV] complex and following phenotypic reversion. A, control cells; B, transformed cells; C, revertants. Magnification x85.

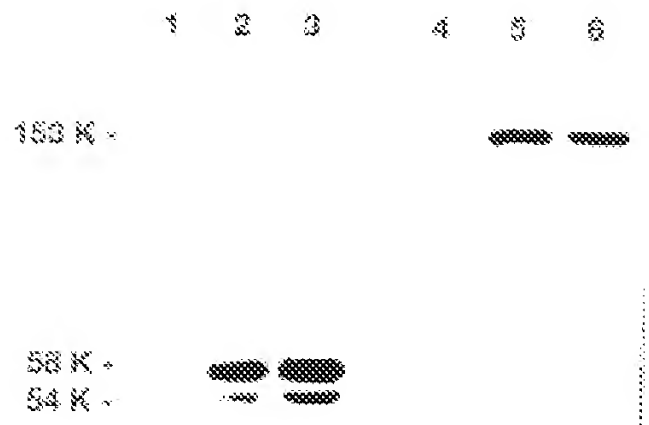


Figure 5. Western blot analysis of MN protein in control NIH3T3 cells, in cells freshly transformed with [pGD.MN+MLV] complex and in the same cells after reversion. Lanes 1-3, reducing gel; lanes 4-6, non-reducing gel. Lanes 1 and 4, extract from control cells; lanes 2 and 5, extract from transformed cells; lanes 3 and 6, extract from revertants. Each lane was loaded with 80 ng protein.

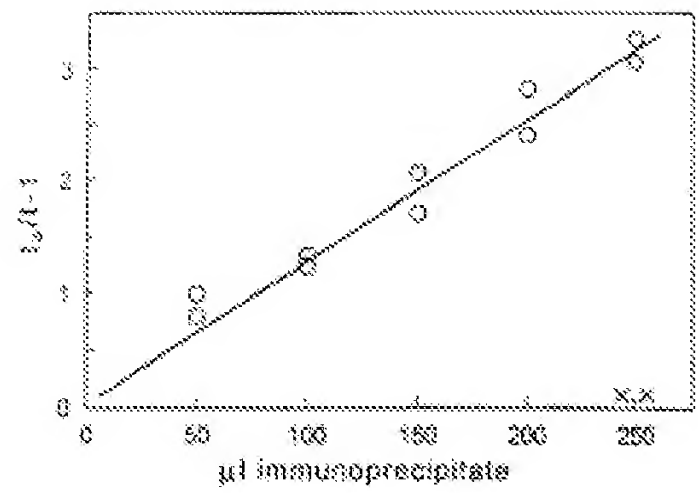


Figure 6. Carbonic anhydrase activity of MN protein. Immunoprecipitate was prepared from: ○, Vero cells infected with vaccinia-MN; X, Vero infected with control vaccinia.

showing signs of apoptosis. Two passages later (Fig. 4C) the cell population totally reverted to original phenotype as a result of frequent emergence of revertants and of their selective advantages (faster growth and a higher efficiency of plating). The revertants appeared to grow to somewhat lower saturation density than original NIH3T3, showing a higher degree of contact inhibition.

Expression of MN protein in cultures parallel with those shown in Fig. 4 was examined by Western blotting (Fig. 5). Control NIH3T3 did not contain any MN protein, while both transformed cells and revertants contained the same amount and the same proportion of 54 and 58 kDa bands of MN protein. In a non-reducing gel, MN protein was present in the form of oligomers of 153 kDa. Consistently, by competition RIA approx. 40 ng MN/mg total protein in both of these cells was found.

CA activity and its inhibition. Since the CA domain represents a considerable part of MN protein (Fig. 1), tests were performed to determine whether it is indeed enzymatically active. Vero cells infected with vaccinia served as a source of MN protein. MN construct, which contained more of the MN protein than other cells was used in the present work. The cells were extracted with RIPA buffer and MN protein was concentrated and partially purified by precipitation with MAb M75 and SAC. The immuno-precipitate was tested for CA activity (Fig. 6). 78 µl of precipitate contained 1 unit of the enzyme. From the extract, the concentration of total proteins and of MN protein was determined; 1 unit of enzyme corresponded to 145 ng of MN protein or to 0.83 mg of total protein. The immuno-precipitate from Vero cells infected with control virus had no enzyme activity.

Activity of MN CA was inhibited by acetazolamide (Fig. 7): 1.53×10^{-6} M concentration of the drug reduced enzyme activity to 50%.

Preliminary tests showed that confluent cultures of HeLa or of NIH3T3 cells tolerated 10^{-5}-10^{-3} M concentration of acetazolamide for 3 days without any signs of toxicity and without any effect on cell morphology. In sparse cultures, 10^{-5} M acetazolamide did not inhibit cell growth, but 10^{-4} M already caused a partial inhibition. Thus, 10^{-5} M aceta-

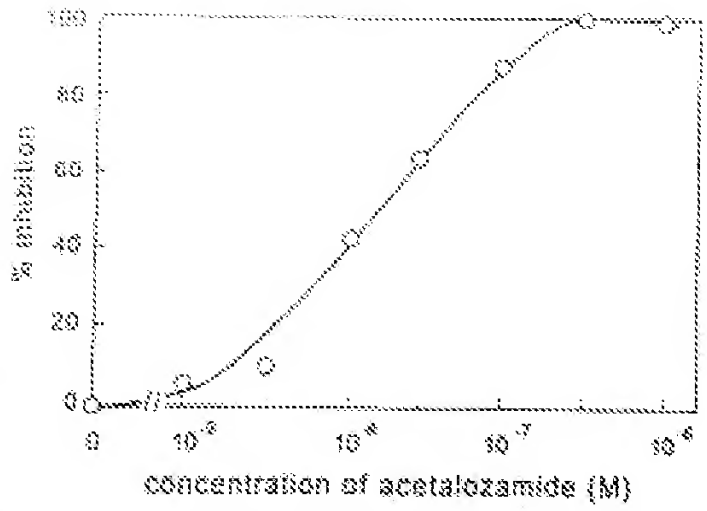


Figure 7. Inhibition of MN carbonic anhydrase activity with acetazolamide.

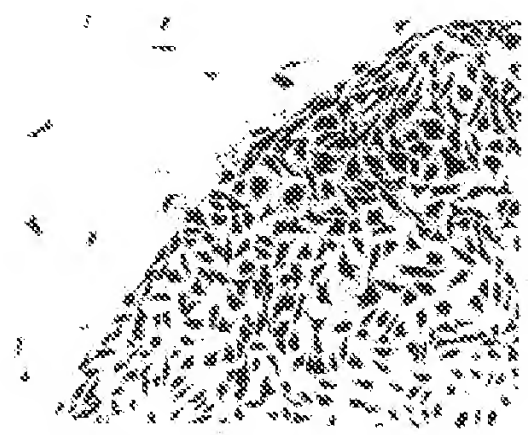


Figure 8. Adhesion of NIH3T3 cells to the area of plastic coated with MN protein. Magnification x40.

zolamide was added to NIH3T3 cells freshly transformed with the [pGD.MN+MLV] complex. After 4 days of incubation, the colonies were fixed and stained. No difference was seen between cells growing in presence or absence of acetazolamide; both were undistinguishable from transformed cells shown on Fig. 4B. Thus, the enzymatic activity of CA is not relevant for the transforming activity of MN protein.

Cell adhesion assay. To determine whether or not MN protein is a new member of CAMs, adhesion assays were performed in plastic bacteriological Petri dishes (not treated for use with tissue cultures). Cells do not adhere to the surface of such dishes, unless it is coated with a binding protein. NIH3T3 cells adhered, spread and grew on patches of adsorbed MN protein; only very few cells attached outside the MN protein coated areas (Fig. 8).

Other variants of the experiment (not shown) demonstrated that NIH3T3 cells adhered and spread on patches of adsorbed collagen I and IV, fibronectin and laminin. The cells did not attach to dots of adsorbed gelatin, FCS or BSA. CGL1, HeLa and Vero cells also adhered to MN protein, but 3 leukemia cell lines showed no adherence. CGL3 cells, strongly expressing MN protein, adhered less efficiently to MN protein dots than CGL1.

Blocking of adsorbed MN protein with an excess of MAb M75 did not abrogate the adhesion of NIH3T3 cells. Also, the presence of 10^{-3} M acetazolamide in the media did not affect the cell adhesion. To confirm the specificity of adhesion, MN protein was absorbed with SAC loaded with MAb M75 (directed to MN) or MAb M67, directed to an unrelated antigen (1), before it was applied to the surface of Petri dishes. Absorption with the SAC-M75 complex totally abrogated the cell binding activity, whereas absorption with SAC-M67 was without any effect.

Discussion

Is MN gene a (proto-) oncogene? Our previous observations and the data presented in this report are consistent with the view that MN gene most likely represents a novel type of oncogene or proto-oncogene. The main arguments for this assumption are: i) MN protein has a strong association with certain types of human carcinomas, and is absent from most of normal tissues (2-7), ii) Cells are morphologically transformed by MN-cDNA, such transformation being characterized by increased cell density, a criss-cross pattern of cell growth (Figs. 2 and 4) and acquisition of the capacity to form colonies in soft agar (8). These features are characteristic for cells transformed with tumor viruses or with cloned oncogenes (23,24).

Adhesion molecules mediate cell-to-cell or cell-to-extracellular matrix binding; they play an essential role in embryogenesis and in cell growth and differentiation. There are reports demonstrating that besides their mechanical function, some CAMs are also involved in signal transduction cascades. Their up-regulation or ectopic expression leads to disruption of normal program of cell differentiation. CAMs are believed to play a role in invasion and metastasis as well as in early steps of carcinogenesis (25,26). Therefore, the present finding of MN being an adhesion molecule appears to us plausible. The development of tumors is generally a multi-step process; in cervical carcinomas, papillomaviruses certainly play an important role (27). Ectopic expression of MN protein could represent an additional step. A third factor participating in genesis of cervical carcinomas is most likely loss or inactivation of tumor suppressor genes.

What suggests the predicted structure of MN protein? The extracellular part of MN protein contains domains homologous to proteoglycans and to CAs. Both of these are known to be engaged in cell-to-cell contacts, or in binding of cells to the extracellular matrix. Receptor protein tyrosine phosphatase β is a cell membrane protein in embryo brains, binding by its CA domain to contactin on the surface of neurons, and by its proteoglycan domain to the glia (11). Another example is vaccinia virus, containing a CA domain in its surface glycoprotein, which is responsible for virus attachment to cellular receptors (28). Both of these structures related to CAs are enzymatically inactive. Due to mutations in the active center, they cannot bind Zn^{2+} ions, but their pocket-like structure was preserved, with potential to accommodate other ligands than CO_2+H_2O.

The CA and proteoglycan domains of MN protein could, similarly to the above mentioned proteins, be involved in cell-to-cell interactions. Its deregulated expression could upset correct communication among cells. Already in 1967, contact inhibition was shown to be mediated by direct

interactions between cells (29), but its molecular mechanism has not been satisfactorily elucidated up to now. MN protein may interfere in signal transmission establishing contact inhibition.

Reversion of tumor cells to normal phenotype was first described in hamster cells transformed with Rous sarcoma virus. The cells changed their chaotic growth back to the original parallel array (30). The *src* oncogene was transcriptionally silenced in segregated revertants. The provirus was shown to be methylated and the methylation is known to prevent transcription (31). Reversion was also found in other tumor cells, which was again due to the methylation of *src* gene (32). The frequency of reversion in these cases was of the order of 10^{-6}/cell/division. Methylation of MN insert was clearly not the cause of reversion in the system presented here, since the revertants contained the MN protein. In some instances, the reversion was explained by mutations of *src* oncogene (33), but in MN, mutation did not account for reversion in MN-transformed cells.

All the MN-transformed clones reverted within 4-5 weeks. A plausible (but not the only) explanation could be that the MN protein expressed in transfected cells, after some interval switches on a suppressor gene(s), which code(s) for a hypothetical 'normalizing' protein. This, in turn, neutralizes transforming activity of MN protein. This postulated suppressor gene is switched off in normal cells, not containing MN protein, and it is lost or inactivated in tumor cells.

Hybridization of HeLa cells with normal fibroblasts (12) indicated that HeLa cells express a critical oncogene, responsible for tumorigenicity in nude mice. This oncogene is counteracted by a tumor suppressor gene (or genes), which is absent or inactive in HeLa cells, but is functioning in fibroblasts. The original HeLa x fibroblast hybrid was non-tumorigenic, but it segregated clones with restored tumorigenicity. Tumorigenic segregants lost chromosome 11, containing the putative suppressor gene.

The MN protein is a candidate for being the product of the critical oncogene; its expression in the hybrids has been shown to correlate with their tumorigenicity (2). The present results indicate that additional mechanisms might exist, which are able to 'heal' a cancerous cell. Understanding the molecular mechanisms of action of MN protein in normal and in tumor cells and elucidating how the reversion works may provide new approaches to cancer therapy.

Acknowledgements

We are grateful to Professor David Baltimore, for the pGD vector, to Professor Claudio Basilico, for valuable advice, to Dr Veronika Simanova and L.L. Lander for help in preparing

3. Liao SY, Brewer C, Zavada J, Pastorek J, Pastorekova S, Manetta A, Berman ML, DiSaia PJ and Stanbridge EJ: Identification of the MN antigen as a diagnostic biomarker of cervical intraepithelial squamous and glandular neoplasia and cervical carcinomas. Am J Pathol 145: 598-609, 1994.
4. Costa MJ, Ndoye A and Trelford JD: MN protein immunolocalization in uterine cervix carcinoma with glandular differentiation. Int J Surg Pathol 3: 73-82, 1995.
5. Liao SY and Stanbridge EJ: Expression of the MN antigen in cervical Papanicolaou smears is an early diagnostic biomarker of cervical dysplasia. Cancer Epidem Biomarker Prev 5: 549-557, 1996.
6. Brewer CA, Liao SY, Wilczynski SP, Pastorekova S, Pastorek J, Zavada J, Kurosaki T, Manetta A, Berman ML, DiSaia PJ and Stanbridge EJ: A study of biomarkers in cervical carcinoma and clinical correlation of the novel biomarker MN. Gynecol Oncol 63: 337-344, 1996.
7. Pastorekova S, Parkkila S, Parkkila A-K, Opavsky R, Zelnik V, Saarnio J and Pastorek J: Carbonic anhydrase IX, MN/CAIX: Analysis of stomach cDNA sequence and expression in human and rat alimentary tracts. Gastroenterology (In press).
8. Pastorek J, Pastorekova S, Callebaut I, Mornon J-P, Zelnik V, Opavsky R, Zatovicova M, Liao SY, Portetelle D, Stanbridge EJ, Zavada J, Burny A and Kettmann R: Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and putative helix-loop-helix DNA binding segment. Oncogene 9: 2877-2888, 1994.
9. Opavsky R, Pastorekova S, Zelnik V, Gibadulinova A, Stanbridge EJ, Zavada J, Kettmann R and Pastorek J: Human MN/CA9 gene, a novel member of the carbonic anhydrase family: structure and exon to protein domain relationships. Genomics 33: 480-487, 1996.
10. Maren TH and Ellison AC: A study of renal carbonic anhydrase. Mol Pharmacol 3: 503-508, 1967.
11. Peles E, Nativ M, Campbell PL, Sakurai T, Martinez R, Lev S, Clary DO, Schilling J, Barnea G, Plowman GD, Grumet M and Schlessinger J: The carbonic anhydrase domain of receptor tyrosine phosphatase β is a functional ligand for the axonal cell recognition molecule contactin. Cell 82: 251-260, 1995.
12. Stanbridge EJ, Flandermeyer RR, Daniels DW and Nelson-Rees WA: Specific chromosome loss associated with the expression of tumorigenicity in human cell hybrids. Somatic Cell Genet 7: 699-712, 1981.
13. Daley QD, Van Etten RA and Baltimore D: Induction of chronic myelogenous leukemia in mice by the P210-*bcr/abl* gene of the Philadelphia chromosome. Science 247: 824-830, 1990.
14. Chakrabarti S, Brechling K and Moss B: Vaccinia virus expression vector: coexpression of beta-galactosidase provides visual screening of recombinant virus plaques. Mol Cell Biol 5: 3403-3409, 1985.
15. Sambrook J, Fritsch EF and Maniatis T: Molecular cloning. A laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, 1989.
16. Kutinova L, Ludvikova V, Simanova V, Otavova M, Krystofova J, Hainz P, Press M, Kunke D and Vonka V: Search for optimal parent for recombinant vaccinia virus vaccines. Study of three vaccinia virus vaccinal strains and several virus lines derived from them. Vaccine 13: 487-492, 1995.
17. Perkus ME, Panicali D, Mercer S and Paoletti E: Insertion and deletion mutants of vaccinia virus. Virology 152: 285-297, 1986.
18. Topp WC: Normal rat cell lines deficient in nuclear thymidine kinase. Virology 113: 408-411, 1981.
19. Brion LP, Schwartz JH, Zavilowitz BJ and Schwartz GJ: Micromethod for the measurement of carbonic anhydrase activity in

24. Macpherson I and Montagnier L: Agar suspension culture for the selective assay of cells transformed by polyoma virus. Virology 23: 291-294, 1964.
25. Piguet R and Power C: The Adhesion Molecule. Academic Press, London, 1993.
26. Rosales C, O'Brien V, Kornberg L and Juliano R: Signal transduction by cell adhesion receptors. Biochim Biophys Acta 1242: 77-98, 1995.
27. Zur Hausen H: Human papillomaviruses in the pathogenesis of anogenital cancer. Virology 184: 9-13, 1991.
28. Maa JS, Rodriguez JF and Esteban M: Structural and functional characterization of a cell surface binding protein of vaccinia virus. J Biol Chem 265: 1568-1577, 1990.
29. Stoker MGP: Transfer of growth inhibition between normal and virus-transformed cells; autoradiographic studies using marked cells. J Cell Sci 2: 293-304, 1967.
30. Macpherson I: Reversion in hamster cells transformed by Rous sarcoma virus. Science 148: 1731-1733, 1965.
31. Searle S, Gillespie DAF, Chiswell DJ and Wyke JA: Analysis of the variations in proviral cytosine methylation that accompany transformation and morphological reversion in a line of Rous sarcoma virus-infected Rat-1 cells. Nucl Acid Res 12: 5193-5210, 1984.
32. Bejtar I, Svoboda J, Geryk J, Fincham VJ and Hak R: High rate of morphological reversion in tumor cell line H-19 associated with permanent transcriptional suppression of the LTR, v-src, LTR provirus. Cell Growth Differ 5: 277-285, 1994.
33. Oppermann H, Levinson AD and Varmus HE: The structure and protein kinase activity of proteins encoded by nonconditional mutants and back mutants in the *src* gene of avian sarcoma virus. Virology 108: 47-70, 1981.

Related Appeals Appendix

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

Paper No. 53

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte JAN ZAVADA, SILVIA PASTOREKOVA and JAROMIR PASTOREK

Appeal No. 2001-1970
Application No. 08/280,190

ON BRIEF

Before WILLIAM F. SMITH, SCHEINER, and GRIMES, Administrative Patent Judges.

GRIMES, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 30, 32, 35-37, and 39-48, all of the claims remaining.

Claims 30, 32, and 36 are representative and read as follows:

30. A method of treating neoplastic disease and/or pre-neoplastic disease in a vertebrate, wherein said disease is associated with abnormal MN gene expression comprising inhibiting the expression of MN gene by administering a MN antisense oligonucleotide in a physiologically acceptable carrier, wherein said MN antisense oligonucleotide is complementary to SEQ ID NO: 5.

32. A method according to Claim 30 wherein said MN antisense oligonucleotide is complementary to the 5' end of the mRNA that is transcribed from the complement of SEQ ID NO: 5.

36. A method according to Claim 30 wherein said MN antisense oligonucleotide is selected from the group consisting of SEQ ID NOS: 3, 4 and 7.

The examiner relies on the following references:

Hoke et al. 5,585,479 Dec. 17, 1996

Wagner, "Gene Inhibition using Antisense Oligodeoxynucleotides," Nature, Vol. 372, pp. 333-335 (1994)

Rojanasakul, "Antisense Oligonucleotide Therapeutics : Drug Delivery and Targeting," Advanced Drug Delivery Reviews, Vol. 18, pp. 115-131 (1996)

Gura, "Antisense Has Growing Pains," Science, Vol. 270, pp. 575-577 (1995)

Reynolds, "First Antisense Drug Trials Planned In Leukemia," Journal of the National Cancer Institute, Vol. 84, pp. 288-289 (1992)

Stein et al., "Antisense Oligonucleotides as Therapeutic Agents – Is the Bullet Really Magical ?" Science, Vol. 261, pp. 1004-1012 (1993)

Wu-Pong, "Oligonucleotides: Opportunities for Drug Therapy and Research," Pharmaceutical Technology, Vol. 18, pp. 102-114 (1994)

Stull et al. (Stull), "Antigene, Ribozyme and Aptamer Nucleic Acid Drugs : Progress and Prospects," Pharmaceutical Research, Vol. 12, pp. 465-483 (1995)

Milligan et al., "Current Concepts in Antisense Drug Design," Journal of Medicinal Chemistry, Vol. 36, No. 14, pp. 1923-1937 (1993)

Branch, "A good antisense molecule is hard to find," TIBS, Vol. 23, pp. 45-50 (1988)

Tseng et al., "Antisense Oligonucleotide Technology in the Development of Cancer Therapeutics," Cancer Gene Therapy, Vol. 1, No. 1, pp. 65-71 (1994)

Miller et al., " Gene Transfer and Antisense Nucleic Acid Techniques," Parasitology Today, Vol. 10, No. 3, pp. 92-97 (1994)

James, "Towards Gene-Inhibition Therapy: A Review of Progress and Prospects in the Field of Antiviral Antisense Nucleic Acids and Ribozymes," Antiviral Chemistry & Chemotherapy, Vol. 2, No. 4, pp. 191-214 (1991)

Westermann et al., "Inhibition of expression of SV40 Virus Large T-Antigen by Antisense Oligodeoxyribonucleotides," Biomed. Biochim. Acta, Vol. 48, No. 1, pp. 85-93 (1989)

Orkin et al., "Report and Recommendations of the Panel To Assess the NIH Investment in Research on Gene Therapy," NIH Report on Gene Therapy (1995)

Friedmann, "Overcoming the Obstacles," Scientific American, pp. 96-101 (June 1997)

Mastrangelo et al., "Gene Therapy for Human Cancer: An Essay for Clinicians," Seminars in Oncology, Vol. 23, No. 1, pp. 4-21 (1996)

Weiss, "Upping the Antisense Ante," Science News, Vol. 139, pp. 103-109 (1991)

Claims 30, 32, 35-37, and 39-48 stand rejected under 35 U.S.C. § 112, first paragraph, as nonenabled.

We reverse.

Background

The specification discloses that a "quasi-viral agent having rather unusual properties was detected by its capacity to complement mutants of vesicular stomatitis virus. . . . The quasi viral agent was called MaTu." Page 2. "MaTu was found by the inventors to be a two-component system, having an exogenous transmissible component, MX, and an endogenous cellular component, MN." Id. The "MX" component was later identified as lymphocytic choriomeningitis virus (LCMV). See the specification, page 3.

"[T]he MN component was found to be a cellular gene, showing only very little homology with known DNA sequences. The MN gene was found to be present in the chromosomal DNA of all vertebrates tested, and its expression was found to be strongly correlated with tumorigenicity." Id., pages 2-3. The

specification discloses the full-length MN cDNA sequence. See Figure 15 and SEQ ID NO:5.

The specification discloses that MN was found to be expressed in a variety of tumor cells but not in normal tissues, with the exception of stomach tissue. See id., pages 8-9. "MN antigen was found by immunohistochemical staining to be prevalent in tumor cells. . . . Thus, the MN gene is strongly correlated with tumorigenesis and is considered to be a putative oncogene." Id., page 9.

The specification discloses that "[a]ntisense nucleic acid sequences substantially complementary to mRNA transcribed from MN genes . . can be used to reduce or prevent expression of the MN gene. . . . Such antisense nucleic acid sequences, preferably oligonucleotides, by hybridizing to the MN mRNA, particularly in the vicinity of the ribosome binding site and translation initiation point, inhibits [sic] translation of the mRNA. Thus, the use of such antisense nucleic acid sequences may be considered to be a form of cancer therapy." Pages 92-93.

The specification discloses that non-tumorigenic (CGL1) cells[1] that were transfected with full-length MN cDNA had increased proliferation rates and plating efficiency. See pages 64-65. By contrast, when MN-expressing tumorigenic (CGL3) cells[2] were transfected with an MN antisense construct, "the effect was the opposite of that of the CGL1 cells transfected with the 'sense'

---

[1] See page 121, line 8 ("non-tumorigenic hybrid clone CGL1").
[2] See page 121, lines 6-7 ("Detected was a 1.6 kb MN-specific mRNA only in two tumorigenic segregant clones--CGL3 and CGL4.").

construct. Whereas the transfected CGL1 cells formed colonies several times larger than the control CGL1, the transfected CGL3 cells formed colonies much smaller than the control CGL3 cells." Page 65.

Finally, the specification provides a working example showing inhibition of MN expression in vitro using either of two antisense oligonucleotides complementary to parts of SEQ ID NO:5. See pages 118-120. The two oligonucleotides are referred to as ODN1 (SEQ ID NO:3) and ODN2 (SEQ ID NO:4). The specification discloses that cells treated with ODN1 showed a 40% decrease in MN expression, while cells treated with ODN2 showed a 25-35% decrease. See page 119.

Discussion

The claims are directed to methods of treating neoplastic disease or inhibiting growth of tumor cells, where the disease or tumor cell growth is associated with abnormal MN gene expression, by administering an MN antisense oligonucleotide that is complementary to SEQ ID NO:5. SEQ ID NO:5 is the 1522-base pair, full-length MN cDNA sequence. See the specification, page 27, lines 21-22.

The examiner acknowledged that the claims are "enabl[ed] for a method for inhibiting the growth of a HeLa cell expressing a MN protein in vitro, the method comprising administering a composition comprising SEQ ID NO:3 or SEQ ID NO:4 to the cell so as to inhibit the growth of the cell." Examiner's Answer, page 4. He also acknowledged that the specification provides adequate guidance to enable those skilled in the art to determine whether a particular

cancer is associated with abnormal MN expression. Id., page 25. He concluded, however, that the claims are not enabled throughout their full scope because the specification "does not reasonably provide enablement for methods of treating neoplastic diseases and/or pre-neoplastic disease associated with abnormal MN gene expression, and of inhibiting the growth of a cancer cell that expresses MN protein in vivo." Id.

The examiner's enablement analysis considered several of the Wands factors. See the Examiner's Answer, pages 4-12. In particular, the examiner relied on the following findings:

- The nature of the invention was a "nucleic acid therapy method." Examiner's Answer, pages 5-6.

- "At the time of filing the art recognized antisense therapy as in its infancy and as highly unpredictable." Id., page 6. More specifically, "[d]etermining an effective antisense sequence, and transferring the antisense sequence to adequate numbers of target cells in vivo and getting specific binding between the antisense sequence and the target mRNA in an amount sufficient to produce a beneficial effect in any animal remain[ed] unpredictable at the time the invention was made." Id. The examiner cited several references discussing various problems remaining to be overcome in the field of antisense therapy. See id., pages 6-10.

- The breadth of the claims "encompasses a wide range of antisense sequences, oligo structures, vector types, or compositions employed as therapeutic agents in the claimed antisense therapy methods to treat a wide range of different types of cancer associated with MN expression, in any and/or all vertebrate animals including humans." Id., page 10.

- The specification provides working examples showing in vitro inhibition of tumorigenic cell growth and inhibition of MN gene expression. Id., page 11. However, the specification does not "demonstrate a reasonable correlation between the in vitro data, i.e., in vi[tro] inhibition of proliferation of a cultured tumorigenic human cell line (CGL3 cells) by direct injection of plasmids

> expressing the antisense nucleic acids molecules of SEQ ID NO:3 of SEQ ID NO:4, and the [claimed method]." Id., page 5.
>
> - Finally, the examiner found that the specification "failed to address" many of the issues that have hampered development of antisense therapeutic methods, including "stability of antisense nucleic acids in an in vivo environment . . .; the ability of a chosen antisense nucleic acid to reach and enter the target cell in vivo; . . . and specificity of an administered nucleic acid . . . to generate a desired therapeutic effect." Id., pages 11-12.

The examiner concluded that "the specification is non-enabling in view of the complex and unpredictab[e] nature of the subject matter, the lack of description and working examples which are correlating to the full scope of the claimed subject matter, the lack of guidance provided as to the selection of essential combination of parameters which would result in an effective therapeutic composition, and the amount of undue experimentation required to practice the full scope of the claimed invention." Examiner's Answer, page 12.

Appellants argue that the examiner has not made out a prima facie case of nonenablement, for several reasons. Appellants argue that the claims are relatively narrow, since the claims are limited to antisense sequences that are complementary to part of SEQ ID NO:5, and the specification provides an in vitro screening method that would enable those skilled in the art to select therapeutically useful antisense oligonucleotides. See the Appeal Brief, pages 24-25.

Appellants also argue that methods of administering therapeutic oligonucleotides were known in the art, as were methods for determining appropriate dosages. See the Appeal Brief, pages 17-18. Appellants argue that

the references cited by the examiner focus on optimization of antisense therapy to the point that it would be ready for clinical application. This standard, Appellants argue, is higher than what is required for enablement. See the Appeal Brief, pages 26-27. Appellants point to the approval of clinical trials using antisense oligonucleotides as evidence of enablement. See id., pages 29-30.

In support of each of the above arguments, Appellants cite declaratory evidence they submitted during prosecution. See the declaration submitted under 37 CFR § 1.132 by Dieter Cotter Gruenert (Paper No. 27, filed July 17, 1997), Appendix II to the Appeal Brief. The examiner disputes the probative value of the declaration, because "[s]ections 3, 4, 5, 6, and 8 as to the in vitro data . . . do not provide factual evidence to indicate that antisense nucleic acid therapy to treat all tumor bearing vertebrate animals . . . is reasonabl[y] predictive at the time the invention was made, nor do the sections provide factual evidence to demonstrate an extrapolation from the guidance and/or in vitro data disclosed in the as-filed application to the entire scope of the claimed invention." Examiner's Answer, pages 24-25.

"When rejecting a claim under the enablement requirement of section 112, the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement. If the PTO meets this burden, the burden then shifts to the

applicant to provide suitable proofs indicating that the specification is indeed enabling." In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993).

"[E]nablement requires that the specification teach those in the art to make and use the invention without 'undue experimentation.' That some experimentation may be required is not fatal; the issue is whether the amount of experimentation required is 'undue.'" In re Vaeck, 947 F.2d 488, 495, 20 USPQ2d 1438, 1444 (Fed. Cir. 1991) (citation omitted, emphasis in original). "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Those considerations, see id., are well known; we need not repeat them here.

In this case, we agree with Appellants that the examiner has not shown that undue experimentation would have been required to practice the claimed method. The examiner's concerns, and the evidence cited in support of the rejection, are mainly directed to sources of unpredictability and experimentation involved in antisense therapy in general, rather than the claimed method in particular. Granted, the examiner's references show that (at least as of 1992) antisense therapy techniques, as a group, required further experimentation before they would be ready for clinical application. This showing, however, is not enough to support a rejection of the instant claims for nonenablement.

First, we agree with Appellants that a therapeutic method need not be ready for clinical application in order to be enabled. See In re Brana, 51 F.3d 1560, 1567, 34 USPQ2d 1436, 1442 (Fed. Cir. 1995): "Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans."[3] The references cited by the examiner seem to focus on the clinical application of antisense therapeutics. For example, the examiner cited Stull's statement that "nucleic acid drugs must overcome several formidable obstacles before they can be widely used as therapeutics." Examiner's Answer, page 9. The examiner also cited Wagner as providing "evidence that antisense was . . . unpredictable at the time the invention was made." Examiner's Answer, page 9.[4]

While the references cited by the examiner provide evidence that antisense therapy, in general, was not ready for broad clinical application in the early 1990s, such evidence is not enough to show nonenablement. What is needed is evidence or sound scientific reasoning that undue experimentation would have been required to carry out the claimed methods. The claims are

---

[3] Although the Brana court referred to "usefulness," the rejection on appeal was based on 35 U.S.C. § 112, first paragraph. See 51 F.3d at 1564, 34 USPQ2d at 1439.

[4] The examiner appears to have evaluated the enablement of the claims as of 1992, based on the filing date of the grandparent application of the present application. However, according to Appellants, the present application is a continuation-in-part of 08/188,093, filed Dec. 30, 1993, which was a continuation-in-part of 07/964,589, filed Oct. 21, 1992. Since the present application does not have the same disclosure as the earlier-filed applications, it is not necessarily entitled to § 120 benefit based on those earlier applications. However, the claims have not been rejected over intervening art, so we need not consider whether the instant claims are entitled to § 120 benefit. We will limit our analysis to whether the claims on appeal would have been enabled by the disclosure of the present application, in light of the state of the art as of this application's 1994

variously directed to methods of "treating neoplastic disease and/or pre-neoplastic disease", "inhibiting growth of a vertebrate cancer cell", or "inhibiting the expression of a MN gene." Thus, the claims at least encompass methods that require some degree of therapeutically beneficial effect. That standard, however, is more lenient than what is required for clinical application. See, e.g., Brana, 51 F.3d at 1568, 34 USPQ2d at 1442 ("On the basis of animal studies, and controlled testing in a limited number of humans (referred to as Phase I testing), the Food and Drug Administration may authorize Phase II clinical trials. . . . Authorization for a Phase II study means that the drug may be administered to a larger number of humans, but still under strictly supervised conditions. The purpose of the Phase II study is to determine primarily the safety of the drug . . . as well as its potential efficacy under different dosage regimens.").

In this case, we have no fact-based explanation from the examiner focused on the claimed methods, as opposed to antisense therapy as a general field, to establish that the instant claims are nonenabled. In addition, it is well-established that the amount of experimentation that is considered "undue" varies from one field to another. See, e.g., Wands, 858 F.2d at 737, 8 USPQ2d at 1404 (factors relating to undue experimentation include quantity of experimentation necessary, nature of the invention, and relative skill of those in the art).

---

filing date. Cf. Reiffin v. Microsoft Corp., 214 F.3d 1342, 1346, 54 USPQ2d 1915, 1917-18 (Fed. Cir. 2000).

In this case, the evidence shows that the FDA had approved several clinical trials of antisense drugs by 1994. For example, Reynolds disclosed that "the first approval from the Food and Drug Administration to test an antisense drug on patients" came in January 1992. See page 288, left-hand column. Wu-Pong reported that, in 1994, "several ON [oligonucleotide] drug candidates are currently being tested in clinical trials." Page 110 (citing Alper, "Oligonucleotides Surge into Clinical Trials," Bio/Technology, Vol. 11, p. 1225 (1993)). Wagner, also in 1994, stated that "[c]linical trials are now in progress to evaluate the therapeutic potential of antisense ODNs [oligodeoxynucleotides] in several human diseases, including myelogenous leukaemia, and infection by human immunodeficiency virus-1, cytomegalovirus (CMV) and human papillomavirus." Page 333, left-hand column (citing references published in 1993 and 1994).

The approval by the FDA of clinical trials before and contemporaneous with the filing date of the instant application provides evidence that those skilled in the art of antisense methods regularly applied therapeutic techniques to human patients, despite the problems remaining to be overcome before the techniques could be widely applied clinically. Thus, the antisense protocols cited by Reynolds, Wu-Pong, and Wagner provide evidence that those practicing antisense techniques would not have considered the obstacles cited by the examiner to be a barrier to applying antisense therapies in human patients, and therefore, that those obstacles would not have been considered to be a source of undue experimentation in this field. There is no evidence in the record that the claimed antisense-based methods would have been likely to involve excessive

experimentation when considered relative to other antisense-based therapeutic methods.

Finally, we must also disagree with the examiner concerning the probative value of the Gruenert declaration. Dr. Gruenert declared that the in vitro results provided in the specification were reasonably predictive of in vivo efficacy. See ¶¶ 3-5. Dr. Gruenert also declared that the published literature at the time of filing would have supported an expectation of success in using MN antisense oligonucleotides to inhibit MN gene expression in vivo. See ¶ 6. Finally, Dr. Gruenert declared that the screening procedure disclosed in the specification, together with the published literature at the time of filing, would have enabled those skilled in the art to identify therapeutically useful oligonucleotides. See ¶¶ 7-8.

Of course, "Appellant's opinion on the ultimate legal issue is not evidence in the case. . . . [However,] some weight ought to be given to a persuasively supported statement of one skilled in the art." In re Lindell, 385 F.2d 453, 155 USPQ 521, 524 (CCPA 1967) (emphasis added). Here, the conclusions set out in the Gruenert declaration were well-reasoned and supported by evidence, either in the specification or in cited prior art references.

The lack of "factual evidence", as the examiner put in, in addition to that provided in the specification, is not a fatal flaw in a Rule 132 declaration. Declaratory evidence can support patentability in a number of ways. In this case, the declaratory evidence was presented to show how the guidance provided by the specification, combined with the state of the art, would have been viewed by

a person of skill in the relevant field. The enablement requirement is determined, of course, from the perspective of those skilled in the art. See Amgen, Inc. v. Hoechst Marion Roussel, Inc., 314 F.3d 1313, 1335, 65 USPQ2d 1385, 1400 (Fed. Cir. 2003), ("[T]he [enablement] requirement is satisfied if, given what they already know, the specification teaches those in the art enough that they can make and use the invention without 'undue experimentation.'").

The examiner appeared to start from the position that the claims were nonenabled, and then evaluate the declaration for whether it provided additional "factual evidence" in rebuttal. This approach is erroneous. "If a prima facie case is made in the first instance, and if the applicant comes forward with reasonable rebuttal, whether buttressed by experiment, prior art references, or argument, the entire merits of the matter are to be reweighed." In re Hedges, 783 F.2d 1038, 1039, 228 USPQ 685, 686 (Fed. Cir. 1986). See also In re Rinehart, 531 F.2d 1048, 1052, 189 USPQ 143, 147 (CCPA 1976):

> When prima facie obviousness is established and evidence is submitted in rebuttal, the decision-maker must start over. . . . An earlier decision should not . . . be considered as set in concrete, and applicant's rebuttal evidence then be evaluated only on its knockdown ability. Analytical fixation on an earlier decision can tend to provide that decision with an undeservedly broadened umbrella effect. Prima facie obviousness is a legal conclusion, not a fact. Facts established by rebuttal evidence must be evaluated along with the facts on which the earlier conclusion was reached, not against the conclusion itself.

While Hedges and Rinehart were addressed specifically to the issue of obviousness, the same evaluation applies to any patentability determination.

Even if a prima facie case is made out, when evidence is submitted in rebuttal, all

of the evidence of record must be considered in deciding whether the rejection is still viable. In this case, Appellants provided evidence showing that those skilled in the art would have viewed the guidance provided by the specification and the state of the art differently than the examiner viewed it. At that point, it was incumbent on the examiner, if he decided to maintain the rejection, to explain why a hypothetical "person of ordinary skill" was more likely to share his view of the evidence than that of Appellants' declarant. That was not done.

Thus, we conclude that the examiner has not shown that the amount of experimentation required to practice the instant claims would have been considered undue by those skilled in the art of antisense methods. The rejection for nonenablement is reversed.

Other Issues

As noted above (see footnote 4), we take no position on whether the instantly claimed invention would have been enabled by the disclosure in this application's parent or grandparent applications, combined with the state of the art in 1993 or 1992. Thus, if intervening prior art exists that would anticipate or render obvious the instant claims, the examiner should determine whether the instant claims are entitled to the benefit of the earlier-filed applications under 35 U.S.C. § 120. That is, the examiner should determine the effective filing date of the instant claims. If the effective filing date is later than any prior art that would anticipate the claims or render them obvious, a rejection under 35 U.S.C. § 102 or 35 U.S.C. § 103 may be appropriate.

Summary

The rejection for nonenablement is not supported by a preponderance of the evidence in the record. We therefore reverse the rejection under 35 U.S.C. § 112, first paragraph.

REVERSED

William F. Smith
Administrative Patent Judge

Toni R. Scheiner
Administrative Patent Judge

Eric Grimes
Administrative Patent Judge

) BOARD OF PATENT
) APPEALS AND
) INTERFERENCES

EG/dym